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A FURTHER STUDY OF THE EXCESS OXYGEN METHOD FOR THE DETERMINATION OF THE BIOCHEMICAL OXYGEN DEMAND OF SEWAGE AND INDUSTRIAL WASTES.[1]

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The biochemical oxygen demand of water polluted with sewage or industrial wastes may be defined as the milligrams of oxygen per liter of water required for the stabilization of its organic matter by aerobic bacterial action. There are three methods available for the determination of this quantity, viz, the "excess nitrate" (Lederer) method, the relative stability method, and the excess oxygen method. Reasons for the adoption of the excess oxygen method in studies conducted in connection with the investigation of methods for purifying industrial wastes and sewage were given in a previous article.[1]

The "excess nitrate" method is inapplicable to the determination of the very small oxygen demand values which obtain in the case of highly polluted streams or fairly good effluents. If dissolved oxygen is present it is not always possible to prophesy whether this method should be used or the sample incubated straight. Standard Methods (A. P. H. A.) (1917) recommends that in such cases the "excess nitrate" and excess oxygen methods be employed alongside. Dilutions are dispensed with in the "excess nitrate" method—a strong point in its favor, which, however, is largely offset by the determinations of the residual nitrites and nitrates. The method is open to the further objections that it is coupled with constants which do not necessarily hold for industrial wastes and that it can not be used to measure organic matter in a state of oxidation only slightly lower than that of nitrates, e. g., free ammonia and nitrites and possibly other forms of oxidizable matter that will eventually abstract definite amounts of oxygen from a stream.

Stability numbers can be used in deriving quantitative results if the initial dissolved oxygen is determined as in the case of the excess oxygen method. Since the oxygen supply is allowed to go

[1] The Determination of the Biochemical Oxygen Demand of Industrial Wastes and Sewage, by Emery J. Theriault, Sanitary Bacteriologist, and Harry B. Hommon, Sanitary Engineer, United States Public Health Service. This paper was published as an appendix to Public Health Bulletin No. 97, and will appear with the present report in reprint form.

to depletion, all titrations after incubation are dispensed with. This method was not used, however, since it could not be stated at the time that the constant involved in the derivation of the stability numbers also held for industrial wastes. Moreover, as will appear later, results based on short periods of incubation are likely to be misleading when this method is used.

Inasmuch as the "excess nitrate" and the relative stability methods had to be calibrated against the excess oxygen method, an attempt was made to develop an easily workable and efficient excess oxygen method that would take the place of the methods referred to above. There appeared to be difficulties and uncertainties in the practical application of the excess oxygen method that militated against its use in spite of its closer simulation of natural conditions. A technique was developed which simplifies the determination considerably, gives reliable results, and compares very favorably in the amount of laboratory work required with any method so far proposed. Based on a series of over 400 tests, it was shown[2] that the reliability of the biochemical oxygen demand values derived by incubating samples of waste or sewage with excess oxygen was well within 5 per cent, or practically within the error of titration. In this article it is intended to present, first, some deductions based on the reliability of the excess oxygen method as developed in this laboratory, and, second, to show what relation 24-hour and 5-day oxygen demand values bear to each other and to the 10-day oxygen demand.

In the article referred to above it was shown quite conclusively that a strict proportionality exists between the amount of oxygen used up during an incubation test and the amount of waste taken for the test, and it was also shown that this proportionality is quite independent of the dilution used. This conclusion has an important application in the study of stream pollution, since wastes discharged into streams are, in general, oxidized in a very highly diluted condition. If it were true that the biochemical oxygen demand of a waste varies with its dilution, being considerably higher in the higher dilutions,[3] it would be logical to assume that a waste diluted 100,000 times, on being added to a stream, would absorb many times the amount of oxygen indicated by a laboratory test on a 1 per cent concentration. The fact that the amount of oxygen required for the stabilization of a waste is independent of the dilution furnishes a scientific basis for the assumption that the results of laboratory studies can be used in gauging the effect of a given waste on the deoxygenation of a stream.

[2] See footnote, page 1087.

[3] University of Illinois Bulletin, Water Survey Series No. 13, vol. 14.

Another question of fundamental importance in the study of stream pollution is the rate at which oxygen is taken up from a stream, since, other things being equal, this rate determines the point at which nuisance would occur. Since industrial wastes constitute a very important factor in the pollution of streams, it becomes desirable to determine the rate at which they absorb oxygen when diluted with water containing dissolved oxygen. This rate would have an important bearing in fixing the responsibility for the creation of a nuisance due to the overloading of the oxidizing capacity of a stream. The rate at which sewage is oxidized has already been determined by Phelps,[4] and has been used by him in deriving the relative stability numbers for sewage. These numbers were derived on the valid assumption that bacterial oxidation processes follow the law of a monomolecular reaction. This equation, in the integrated form, and for the particular case of the oxidation of organic matter by bacteria, is

$$\frac{(a-x)}{(a)} = 1 - K^t \tag{1}$$

where $(a-x)$ is the organic matter used up during t days,
(a) is the organic matter present at the start,
and (K) is a constant estimated by Phelps to be 0.794 in the case of sewage incubated at 20° C.

Since the organic matter is to be measured in terms of the oxygen required to stabilize it, the term $\frac{(a-x)}{(a)}$ in equation (1) may be replaced by the equivalent ratio

$$\frac{\text{Dissolved oxygen used up during the time } t}{\text{Total biochemical oxygen demand}} = S \tag{2}$$

The stability of sewage is defined as the above ratio. When a relative stability of 100 is adopted as a standard, equation (1) becomes

$$S = 100(1 - 0.794^t) \tag{3}$$

In the derivation of this equation it is assumed that the oxidizing bacteria and the organic matter to be oxidized are in equilibrium from the very start of the incubation. Furthermore, no account is taken of the purely chemical part of the oxidation, i. e., the initial oxygen demand. This purely chemical part of the oxygen requirement is generally small in comparison with the 5 or 10 day biochemical oxygen demand, and it is satisfied during the first few hours of a test. In some cases, however, its value may be large in comparison

[4] Phelps, Earle B., U. S. Geological Survey Water-Supply Paper No. 229.

with the 1-day oxygen demand. The assumption that oxidizing bacteria and organic matter are in equilibrium is approximately true where dissolved oxygen is present in the sample under examination.

In the case of wastes which must be diluted before the stability test can be applied, time should obviously be allowed for the development of aerobic bacterial activity. The term t in equation (3) in such cases should be replaced by the term $(T-L)$, where T is the observed time of incubation and L is the lag in the establishment of full bacterial activity under the conditions of the test. This lag is an indeterminate factor, and, as will appear later on, may vary from 0 to 18 hours. Other things being equal, the disturbing effect of this delay in the establishment of bacterial equilibrium would be greater for the shorter periods of incubation. These objections are not valid when the stability numbers are used for their original purpose, since the same lag in the establishment of bacterial activity may be expected when wastes are discharged into a stream and samples possessing dissolved oxygen do not have any initial oxygen demand. When the stability numbers are used to determine the ultimate biochemical oxygen demand of a waste requiring dilution it is apparent, however, that any results based on 1- or 2-day periods of incubation may be in serious error.

With the above reservations, the time t in equation (3) is known and the amount of oxygen available at the start can be readily determined. The amount used up during the time t can be estimated by one of two methods:

(*a*) The Relative Stability Method.—By adding methylene blue to the sample and noting the time required for its decolorization. In this case the amount of oxygen used up is that which was present at the start of the test.

(*b*) The Excess Oxygen Method.—The amount of oxygen present in the sample after a definite period of incubation is determined and the amount used up during that time is found by difference.

It will be noted that in the first method the oxygen supply is allowed to become depleted, whereas in the second method depletion, of necessity, must not have been reached. Before comparing the two methods it must, therefore, be established that the rate at which oxygen is used up is the same, whether or not an appreciable amount of oxygen is present. This has been shown to be the case[5], and a logical basis, therefore, exists for comparing the two methods.

[5] See footnote, page 1087.

The following table was computed from the equation $S=100\ (1-0.794^t)$, the manner of computing the stability for a 1-day period being as follows:

$$S=100\ (1-0.794^1)=20.6.$$

The stability numbers given in the table are those corresponding to 1-, 5-, and 10-day periods of incubation. In order to estimate the effect of a lag of from 12 to 18 hours in the establishment of bacterial equilibrium, periods differing from these by 12 or 18 hours are also included.

TABLE 1.—*Relative stability numbers for various periods of time.*

Time in days	0.25	0.5	1.0	4.25	4.5	5.0	9.25	9.5	10.0
Stability	5.60	10.9	20.6	62.50	64.6	68.5	88.2	88.9	90.1

Given the relative stability numbers and the biochemical oxygen demand values corresponding to two different periods of time, it is possible to connect the relative stability numbers with the excess oxygen method in the following manner:

$$S_1=\frac{\text{Oxygen demand during } t_1 \text{ days}}{\text{Total oxygen demand}} \tag{4}$$

$$S_2=\frac{\text{Oxygen demand during } t_2 \text{ days}}{\text{Total oxygen demand}} \tag{5}$$

Dividing equation (4) by equation (5):

$$\frac{S_1}{S_2}=\frac{\text{Oxygen demand during } t_1 \text{ days}}{\text{Oxygen demand during } t_2 \text{ days}} \tag{6}$$

For sewage, therefore, the oxygen demand values over different periods of time are in the same ratio as the corresponding stability numbers. The same relation should hold true for industrial wastes if sewage and industrial wastes are oxidized at the same rate. The ratios of the stability numbers given in Table 1 are recorded in Table II, and the corresponding ratios for a number of the more important industrial wastes are given in Table III. These ratios are the factors to be applied to oxygen demand values for a given period of incubation in order to estimate the oxygen demand for a different period. In Table II under the headings "Five-day to one-day," "Ten-day to one-day," and "Ten-day to five-day," the ratios of stabilities differing from 1-, 5-, and 10-day periods by 12 or 18 hours are also given.

TABLE II.—*Ratios of the stability numbers for sewage for various periods.*

S_1	S_2	$\frac{S_2}{S_1}$ = Ratio of the stabilities.
FIVE-DAY TO ONE-DAY.		
20.6	68.5	3.3 = Ratio of 5-day to 1-day stability number.
10.9	64.6	5.9 = Ratio of 4.5-day to 12-hour stability number.
5.6	62.5	11.2 = Ratio of 4.25-day to 6-hour stability number.
TEN-DAY TO ONE-DAY.		
20.6	90.1	4.4 = Ratio of 10-day to 1-day stability number.
10.9	88.9	8.2 = Ratio of 9.5-day to 12-hour stability number.
5.6	88.2	15.8 = Ratio of 9.25-day to 6-hour stability number.
TEN-DAY TO FIVE-DAY.		
68.5	90.1	1.32 = Ratio of 10-day to 5-day stability number.
64.6	88.9	1.38 = Ratio of 9.5-day to 4.5-day stability number.
62.5	88.2	1.41 = Ratio of 9.25-day to 4.25-day stability number.

In Table II it will be noted that if bacteria and organic matter are in equilibrium at the start of a test, the ratio of the 5-day to the 1-day stability numbers is 3.3. If a lag of 12 hours is allowed before equilibrium is established, the ratio becomes 5.9. Similarly, the ratios of 10-day to 1-day stability numbers may vary from 4.4 to 15.8 If 12 hours elapse before bacterial equilibrium is established, the results of a 24-hour incubation test should be correlated with the stability number corresponding to 12 hours and not with the 24-hour stability number. Where 5- and 10-day stability numbers are involved, the variation in the value of the ratios is small, since, in this case, a difference of 12 or 18 hours is quite negligible.

TABLE III.—*Ratios of the biochemical oxygen demand values of various industrial wastes for different periods of time.*

UNTREATED WASTES.

Industry.	Source of samples.	Number of tests made.	5-day to 1-day.	10-day to 1-day.	10-day to 5-day.
			$R_1 \pm A.D.$	$R_2 \pm A.D.$	$R_3 \pm A.D.$
Breweries	Mixed wastes	113	3.9±0.1	4.8±0.2	1.2±0.0
Tomato canning	do	7	5.0±1.1	6.0±1.3	1.2±0.0
Tannery (Cincinnati)	Spent tan	10	6.9±1.0	9.0±1.3	1.3±0.0
Do	Beam house	18	4.6±0.5	6.0±0.7	1.3±0.0
Do	Mixed wastes	33	5.8±0.8	7.4±1.0	1.3±0.0
Dairies	do	13	5.4±0.8	6.8±0.9	1.3±0.0
Strawboard	Beater	19	9.3±1.5	13.0±2.2	1.4±0.0
Do	Machine	18	4.5±0.4	6.6±0.6	1.4±0.0
Do	Mixed wastes	15	6.6±0.9	9.8±1.5	1.4±0.0
Abattoirs	do	64	4.0±0.2	5.5±0.3	1.4±0.0

Table III.—*Ratios of biochemical oxygen demand values of various industrial wastes for different periods of time*—Continued.

Industry.	Source of samples.	Number of tests made.	5-day to 1-day. $R_1 \pm A.D.$	10-day to 1-day. $R_2 \pm A.D.$	10-day to 5-day. $R_3 \pm A.D.$
SLIGHTLY TREATED WASTES.					
Tomato canning	Imhoff tank	7	5.8±0.7	6.5±0.8	1.1±0.0
Tannery (Cincinnati)	Settled waste	40	4.0±0.2	5.5±0.3	1.3±0.0
Tannery (Luray, Va.)	do	15	2.7±0.3	3.1±0.3	1.5±0.0
SEMIPURIFIED WASTES.					
Tomato canning	Cinder filters	14	3.6±0.3	4.5±0.6	1.3±0.0
Tannery (Cincinnati)	do	40	4.3±0.2	6.2±0.4	1.5±0.0
Do	Coke filter	45	3.9±0.2	5.9±0.3	1.5±0.0
Tannery (Luray, Va.)	Cinder filter	14	3.5±0.6	4.6±0.8	1.2±0.0
PURIFIED WASTES.					
Tomato canning	Sand filters	17	5.0±0.4	7.8±0.6	1.6±0.1
Tannery (Cincinnati)	Filter, No. 5	19	3.8±0.3	5.9±0.6	1.7±0.1
Do	Filter, No. 6	27	3.6±0.2	6.3±0.5	1.7±0.1
Tannery (Luray, Va.)	Sand filters	28	3.1±0.3	4.3±0.4	1.4±0.0
Filtration	Tap water	15	3.2±0.4	5.4±0.5	1.5±0.1
CREAMERY WASTES.					
Creamery	Mixed wastes	19	4.1±0.6	5.8±0.9	1.4±0.0
Do	Settled waste	19	7.0±1.6	9.3±2.3	1.5±0.0
Do	Sand filter, No. 1	12	3.7±0.3	7.5±0.8	2.1±0.2
Do	Sand filter, No. 2	24	4.3±0.4	10.0±1.0	2.0±0.1

The ratios for the biochemical oxygen demand values of a variety of the more important industrial wastes are given in Table III. The wastes are grouped according to the degree of their oxidation with the exception of the values given for creamery waste. The values for this type of waste were obtained at another laboratory using a different technique and are not strictly comparable with the rest of the values in the table. The manner of deriving the average ratios given in this table was as follows:

$$A_1, A_2, A_3 \ldots A_n = \text{1-day oxygen demand results.}$$
$$B_1, B_2, B_3 \ldots B_n = \text{5-day oxygen demand results.}$$
$$C_1, C_2, C_3 \ldots C_n = \text{10-day oxygen demand results.}$$
$$n = \text{Number of tests covering 3 periods.}$$

The ratios between the values at 3 different periods were computed for each set of results obtained and the mean value of the ratios, *R*, was computed from the following expression:

$$R_1 = \frac{\frac{B_1}{A_1} + \frac{B_2}{A_2} + \frac{B_3}{A_3} + \cdots + \frac{B_n}{A_n}}{n} = \text{Mean value of 5-day to 1-day ratio.}$$

Given the mean value of a ratio, the deviation of an individual ratio from this value is, in the case of 5- and 1-day ratios:

$$d_1 = R_1 - \frac{B}{A}$$

The average deviation, *a. d.*, is the sum of the individual deviations divided by the number of tests made:

$$a.\ d. = \frac{d_1 + d_2 + d_3 + \cdots + d_n}{n}$$

and the deviation of the mean, *A. D.*, is given by the expression:

$$A.\ D. = \frac{a.\ d.}{n^{1/2}}$$

In the last column of Table III, under the heading "Ten-day to five-day," are given the ratios found experimentally to hold true for the wastes in question. In this column R_3 is the mean of the individual ratios and *A. D.* is a measure of the precision of R_3. In most cases the deviation of R_3 was less than 0.05 and the term *A. D.* was recorded simply as 0.0. With the exception of the values grouped under "Purified wastes" the ratios in this column are in very close agreement and do not depart appreciably from a value of 1.3 or 1.4. In fact if all values given in this column are averaged, an average ratio of 1.38 ± is obtained. The average ratio for the "Untreated," "Slightly treated," and "Semipurified" wastes considered separately is 1.33 ± 0.0. The "Purified wastes" considered separately give an average ratio of 1.48 ± 0.1. The value of 1.33 is practically identical with that given in Table II derived from Phelps's constant of 0.794 for sewage.

It will be noted that the *A. D.* of the values given under "Purified wastes" is greater than that of the less highly oxidized wastes. The depletions from which the values for the "Purified wastes" were obtained were very much smaller than for the other wastes, and the opportunity for experimental error was accordingly much greater. For all practical purposes the deviations are negligible: thus it would make no difference whether a factor of 1.3 or 1.5 were used to compute the 10-day oxygen demand of a sample with a 5-day demand of only 10 p. p. m. Tap water is included in this list, the value derived being 1.5 ± 0.1. The fact that this value so closely approximates that found for raw wastes is a strong indication that the values given for some of the sand filters are too high. In view of the variety of wastes considered, ranging in character from spent tan liquors to dairy waste, and in strength from tap water, with a total oxygen demand of less than 1.0 p. p. m., to abattoir wastes, with oxygen demand values in many

cases of over 10,000 p. p. m., the agreement in this part of the table is very good.

Under the headings "Five-day to one-day" and "Ten-day to one-day" are given the ratios of 5- and 10-day results to 1-day results. At first sight these do not appear to be very concordant. The good agreement between the 5- and 10-day results indicates that the 1-day results are the disturbing factor. The establishment of aerobic conditions in the case of the "Untreated wastes," and the "Slightly treated wastes" involves a change in the bacterial flora, and time must be allowed for aerobes or facultative aerobes to supplant the existing types. Some of the wastes are presumably bactericidal in the undiluted condition. Others have been boiled and are quite sterile. The initial oxygen demand of some of these wastes tends to counterbalance this lag in the establishment of aerobic bacterial activity. This effect is particularly noticeable in the case of the tannery waste samples from Luray, Va. These samples were shipped to the Cincinnati laboratory and analyzed 24 hours or more after being collected. Their initial oxygen demand was considerable. For many wastes, however, e. g., the strawboard wastes and the "Purified wastes," the initial oxygen demand was zero, and the variations in such cases can be ascribed wholly to a lag in the establishment of bacterial equilibrium.

The ratios given under the heading "Five-day to one-day" range from a low value of 2.7 ± 0.3 for settled waste from the Luray tannery, to 9.3 ± 1.5 for beater waste from strawboard mills. Referring to the values given in Table II under the same heading it will be seen that this variation is no greater than might be expected if a lag of from zero to 18 hours is allowed for the establishment of aerobic bacterial activity.

The 5-day to 1-day ratios for the "Semipurified wastes" and the "Purified wastes" range from 3.1 to 5.0, indicating that for these wastes less than 12 hours are required for the establishment of bacterial equilibrium. Tap water, within the limits of experimental error, appears to require very little time for the establishment of uniform bacterial activity. The extreme values for the ratios given under the heading "Ten-day to one-day" in Table III are 3.1 ± 0.3 and 13.0 ± 2.2, whereas in Table II under the same heading the extreme values are 4.4 and 15.8.

The combined effect of the initial oxygen demand and of the delay in the establishment of bacterial equilibrium is to make the results of a 1-day period of incubation rather unreliable for the purpose of estimating the oxygen demand for longer periods of incubation. It is not intended that the values given for the conversion of 1-day results to longer periods of incubation should be used for that purpose. These values have been included in the

table to show the extreme variability of 1-day oxygen demand results as compared with the relatively constant relation obtaining after longer periods of incubation. The reliability of the 5- to 10-day ratio is such, however, that if the 5-day values were to be multiplied by a factor of 1.33 the result would be practically equivalent to the 10-day values actually found by experiment. This indicates that incubation tests may be limited to 5-day periods and suggests the possibility of still further reducing the period of incubation. Since bacterial equilibrium appears to have been established within 18 hours in all cases considered, it is not improbable that the oxygen demand during the second day would bear a very definite relation to the 5- or 10-day oxygen demand. The oxygen demand during the second day could be determined by deducting from the 2-day oxygen demand the value found after one day of incubation. In an assumed case the 10- or 20-day oxygen demand could be computed as follows:

$$\begin{aligned} \text{2-day oxygen demand} &= 200 \\ \text{1-day oxygen demand} &= \underline{\ \ 50} \\ \text{Demand during the second day} &= 150 \\ n\text{th day oxygen demand} &= [50 + (150)\ (\text{factor})] \end{aligned}$$

Experimental data are lacking for the verification of this assumption. Theoretically, the factor to be substituted in the above expression would be $87.5 \div 20.6$, or 4.25, to obtain the 10-day oxygen demand value. If this assumption could be shown to hold true without too great a sacrifice in the accuracy of the results, the period of incubation in the excess oxygen method could be reduced to 2 days.

Summary and Conclusions.

1. Biochemical oxygen demand methods involving the addition of nitrates or the use of stability number relations are open to objections.

2. The excess oxygen method yields very accurate and consistent results with as little labor as, or less labor than, is required by other methods.

3. Laboratory studies of the oxygen demand of sewage and industrial wastes can be applied with confidence to the study of the deoxidizing effect of these wastes on streams, since the amount of oxygen used up in laboratory tests is independent of the dilution used.

4. Phelps's constant for sewage can be used in the study of stream pollution, inasmuch as the rate of sewage oxidation by bacteria is sensibly the same as that for the more important industrial wastes. It follows that industrial wastes and sewage can be compared

directly when their strength is expressed in terms of their biochemical oxygen demand.

5. The close agreement between 5- and 10-day oxygen demand values indicates that the application of a factor of 1.33 to 5-day oxygen demand values is sufficiently accurate for most purposes and that the incubation period need not be extended beyond 5 days.

6. A method whereby the period of incubation might be further reduced to 2 days is suggested.

A NATIONAL INSTITUTE OF INDUSTRIAL PSYCHOLOGY AND PHYSIOLOGY.[1]

In the current number of *The Times Engineering Supplement* (London) a detailed account is given of the proposed National Institute of Psychology and Physiology applied to Industry and Commerce. Among the names of its supporters we note those of Sir Walter M. Fletcher, Sir R. A. Gregory, Mr. W. B. Hardy, Dr. Leonard Hill, Sir Alfred Keogh, Dr. C. S. Myers, Sir E. Cooper Perry, Prof. C. S. Sherrington, and Prof. E. H. Starling. The intention of the founders is to establish a national institute which will investigate the human problems of industry and commerce, occupying a position similar to that held in the domain of physical science by the National Physical Laboratory. It will provide training courses and lectures for those interested in the practical applications of psychology and physiology to the problems of industry and commerce. It will undertake investigations at factories and offices in relation to any special problems—e. g., the conditions necessary to give optimum output, the methods of reducing mental and muscular fatigue, the application of tests by which workers can be selected for the occupations for which they are mentally or physically best fitted, and the conditions which tend to the health, comfort, and welfare generally of the worker. The institute will not be established for profit, and a close relation will be maintained with the Industrial Fatigue Research Board, but overlapping of effort is to be avoided. A Scientific Committee is being formed, consisting of the principal psychologists and physiologists throughout the country interested in such problems, in order to coordinate research work and, so far as possible, to support it by means of grants. We heartily wish success to so impartial and scientific a body of workers in their efforts to deal with important problems, in which a previous medical training is obviously of enormous value.

Editorial Note.—The investigation of problems in industrial physiology, such as are outlined above for the proposed National

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[1] From *The Lancet*, Apri 1 3, 1920, pp. 779–780.